IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of	:	Knipe, et al.
Serial No.	:	08/278,601
Filed	:	July 21, 1994
For	:	Herpesvirus Replication Defective Mutants
Group	:	1817
Examiner	:	Caputa

Assistant Commissioner of Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R.§1.608(b)

I, Min Gao, declare:

1. That prior to September 25, 1990 I worked in the Laboratory of Dr. David Knipe, one of the named inventors of the above-captioned application. My curriculum vitae is attached hereto as Appendix A.

2. That the following is a factual description of experiments performed by me in the United States prior to September 25, 1990.

3. That I was requested to perform these experiments by Dr. David Knipe.

4. That Appendix B attached hereto is a true copy, with dates deleted, of a laboratory notebook page written by me in conjunction with the performance of the experiments performed by me in the United States before September 25, 1990, and that the notebook page of Appendix B accurately reports the following experiments that were performed by me.

5. That the experiments I performed in the United States prior to September 25, 1990 were as follows:

An ICP8 gene internal deletion mutant herpesvirus d301 was propagated and titrated on S-2 cells. S-2 cells can produce the mutant herpesvirus because they express the ICP8 protein from a resident gene upon viral infection, thus they provide the complementing gene product that the mutant protein is missing. A replication defective mutant such as d301 will not however replicate on normal cells such as Vero cells which are routinely used for growth of HSV-1, since they lack an ICP8 gene.

S-2 cells were infected with d301 mutant herpesvirus. After harvesting the propagated virus from the cells, the virus was tested for its ability to replicate by measuring plaque formation in cells infected with the mutant herpesvirus. The mutant herpesvirus ICP8 d301 was tested for plaque formation on S-2 cells and on Vero cells. The results showed that the d301 mutant herpesvirus failed to produce plaques on the Vero cells, even at the lowest dilution tested (10^{-2}). At that same low dilution the S-2 cell monolayer was destroyed by the d301 mutant herpesvirus. At much higher dilutions (10^{-7}) distinct plaques were formed on the S-2 cells, were counted and a viral titer of 1.7×10^{9} was calculated. These results confirmed that d301 is a replication defective ICP8 mutant herpesvirus. The d301 mutant herpesvirus was aliquoted and designated "d301.a _____" stock.

6. That on information and belief, an aliquot of this d301a stock was delivered to the laboratory of Dr. Robert Finberg, one of the named inventors of the above-captioned application prior to September 25, 1990.

7. That the following correlates the above-described experiment to the notebook page provided in Appendix B:

A. The notebook page records the making of a stock of d301.a mutant herpesvirus. This is stated on the top of the page: "Stock of d301.a"

B. Four T-150 tissue culture flasks were each seeded with 10^{7} S-2 cells, passage 14. 4×10^{5} pfu of d301A-1, having a titer of 1.2×10^{7} was added to each T-150 flask. The

cells and virus were incubated for 1 hour at 37°C, the virus was removed and the cells were incubated at 37°C. This is stated on the notebook page at the top:

"①	4-T-150	S-2	p14	10^7 cells/T-150
②	d301A-1	1.2 x 107		_____
③	0.01 pfu	x 4 flasks x 10^7 =		4 x 10^5"
	"10 ml/T-150	37° C /		hr
	Suck off	add 45 ml		37°C"

C. The infected cells were harvested, resuspended, sonicated and the virus-containing suspension was aliquoted. A sample of the propagated mutant herpesvirus was tested for plaque formation. There was no plaque formation in the Vero cells and positive plaque formation in the S-2 cells. The titer of the mutant herpesvirus was 1.7 x 10^9. This is stated on the notebook middle of the page:

"_____ AM Harvest add 12 ml supn, 6 ml milk
....sonicated aliquoted
"d301.a _____"

	10^{-2}	10^{-3}	10^{-7}	10^{-8}
vero	0	0		
S-2 p17			85	6

$85 \times 2 \times 10^7 = 1.7 \times 10^9$"

8. That I hereby declare that all statements made herein are true, and all statements made on information and belief are believed to be true, and further that all statements were made with the knowledge that any willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-identified application or any patent issued thereon.

Date: 8-26-98

Min Gao

APPENDIX A

I. PERSONAL

Name: Min Gao

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II. EDUCATION

School	Date	Major	Degree
Wuhan Univ.	1980	Virology	
Penn. State Univ College of Medicine	1985	Microbiology	Ph.D.

III. EMPLOYMENT HISTORY

Harvard Medical School
Department of Microbiology & Molecular Genetics
Instructor, 1989-1992

Harvard Medical School
Department of Microbiology & Molecular Genetics
Post-doctoral Fellow, 1985-1989

Chinese Academy of Sciences
Wuhan Institute of Virology
Virology Technician, 1974-1976

IV. PRESENT EMPLOYMENT

Bristol-Myers Squibb Pharmaceutical Research Institute
Dept. of Virology
Research Investigator II, 1992 - 1994
Senior research Investigator I, 1994 - 1997
Senior research Investigator II, 1997 - present

VI. PUBLICATIONS

1. Isom, H. C., **Gao, M**. and Wigdahl, B. (1984) Characterization of Guinea Pig Cytomegalovirus DNA. J. Virol., 49:426-436.

2. **Gao, M.** and Isom, H. C. (1984) Characterization of the Guinea Pig Cytomegalovirus Genome by Molecular Cloning and Physical Mapping. J. Virol., 52:436-447.

3. Isom, H. C., and **Gao, M.** (1988) The Pathogenicity and Molecular Biology of Guinea Pig Cytomegalovirus, p.247-266. In G. Darai (ed.), Virus Disease in Laboratory and Captive Animals. Martinus Nijhoff Publishing, Boston.

4. **Gao, M.**, Bouchey, J., Curtin, K., and Knipe, D. M. (1988) Genetical Idetification of a Portion of the Herpes Simplex Virus ICP8 Protein Required for DNA Binding. Virology 163:319-329.

5. **Gao, M.**, and Knipe, D. M. (1989) Genetic Evidence for Multiple Nuclear Functions of the Herpes Simplex Virus ICP8 DNA-Binding Protein. J. Virol. 63:5258-5267.

6. Yin, C-Y., **Gao, M.** and Isom, H. C. (1990) Guinea Pig Cytomegalovirus Immediate Early Transcription. J. Virol. 64:1537-1548.

7. Bush, M., Yager, D. R., **Gao, M.**, Weisshart, K., Marcy, A. I., Coen, D. and Knipe, D. M. (1991) Correct Intranuclear Localization of the Herpes Simplex Virus DNA Polymerase Requires the Viral ICP8 DNA-Binding Protein. J. Virol. 65:1082-1089.

8. **Gao, M.**, and Knipe, D. M. (1991) Potential Role for Herpes Simplex Virus ICP8 DNA Replication Protein in Stimulation of Late Gene Expression. J. Virol. 65:2666-2675.

9. **Gao, M.**, and Knipe, D. M. (1992) Distal Protein Sequences Can Affect the Function of a Nuclear Localization Signal. Mol. Cell. Biol. 12:1330-1339.

10. Thomas, M., **Gao, M**., Knipe, D. M. and Powell, K. (1992) Herpes Simplex Virus DNA Replication: Association Between the Major DNA-Binding Protein and Alkaline Nuclease. J. Virol. 66:1152-1161.

11. **Gao, M.**, and Knipe, D. M. (1993) Intagenic Complementation of Herpes Simplex Virus DNA-Binding Protein Mutants. J. Virol. 67:876-885.

12. **Gao, M.**, DiTusa, S. F., and Cordingley, M. G. (1993) The C-Terminal Third of UL42, a HSV-1 DNA Replication Protein, Is Dispensible for Viral Growth. Virology 194:647-653.

13. Knipe, D. M., de Bruyn Kops, A., **Gao, M.**, and Villarreal, E. (1994) Gene UL29 Encoding the Major DNA-Binding Protein, Infected Cell Protein 8. D. J. McGeoch (ed.) Genes of Herpes Simplex Virus.

14. **Gao, M.**, Matusick-Kumar L., Hurlburt W., DiTusa S.F., Newcomb W.W., Brown J.C., McCann P.J., Deckman I. and Colonno, R. J. (1994) The Protease of Herpes Simplex Virus Type 1 is Essential for Functional Capsid Formation and Viral Growth. J. Virol. 68:3702-3712.

15. Matusick-Kumar, L., and Hurlburt, W., Newcomb W.W., Brown J.C., Weinheimer, S., and **Gao, M.** (1994) Phenotype of Herpes Simplex Virus Protease Substrate ICP35 Mutant. J. Virol. 68:5384-5394.

16. Matusick-Kumar, L., Newcomb W.W., Brown J.C., McCann P.J., Hurlburt, W., Weinheimer, S., **Gao, M.** (1995) The C-terminal 25 amino acids of HSV-1 protease and its substrate ICP35 are involved in the formation of sealed capsids. J. Virol. 69:4347-4356.

17. Matusick-Kumar, L., McCann P.J., Robertson, B., Hurlburt, W., Newcomb W.W., Brown J.C., **Gao, M.** (1995) Release of the Catalytic Domain, N_O, form the HSV-1 Protease is Essential for Viral Growth. J. Virol. 69:7113-7121.

18. Robertson, B., McCann P.J., Newcomb W.W., Brown J.C., Colonno, R. J. and **Gao, M.** (1996) Separate Functional Domains of the HSV-1 Protease: Evidence for Cleavage inside Capsids. J. Virol. 70:4317-4328.

19. Robertson, B., McCann III, P.J., Matusick-Kumar, L., Preston, V. G., and **Gao, M.** (1997) Na, an Autoproteolytic Product of the Herpes Simplex Virus Type 1 Protease, can Functionally Substitute for the assembly protein ICP35. J. Virol. 71:1683-1687.

20. Functional conservation of the alkaline nuclease of HSV-1 and HCMV. **Gao, M.**, Robertson, B., McCann III, P.J., O'Boyle II, Weller, S. K., Newcomb W.W., Brown J.C., Weinheimer, S. P. (in press, Virology).

21. The alkaline nuclease of HSV-1 is absolutely essential for viral replication in a mice model. (in preparation).

22. The Distinct Specificity of the R and M Sites of the HSV-1 Protease. Robertson, B. J., McCann III , P.J., Desai, P., Person, S., and **Gao, M.** (in preparation).

VII. Award:

Bristol-Myers Squibb (1996) "Presidential Award " for the discovery and characterization of the hepatitis B antiviral preclinical lead, BMS 200,475.

APPENDIX B

Stock of d301.9

(1) 1 9-T-150 S-2 P14 10^7 cells/T-150

(2) d301A-1 1.2×10^7

(3) 0.01 Mr x 4 flasks x 10^7 = 4×10^5

$4 \times 10^5 / 1.2 \times 10^7 = 33 \lambda / 40$ ml

10 ml / T150 37 C / hr

Stock off. add 45 ml MEM 0/5

37 C

AM Harvest add 12 ml Supn, 6 ml mill, - 80

Sonicated. aliquot

" d301.9 "

	10^{-2}	10^{-3}	10^{-7}	10^{-8}
vero	0 (many cells)	0		
S-2 P17			85	6

$85 \times 2 \times 10^7 = 1.7 \times 10^9$